DRAFT STUDY DESIGNS FOR ROUTINE
PERINATAL (IN UTERO/LACTATIONAL) EXPOSURE

# I. RANGE FINDING STUDY

## A. PURPOSE

This range finding study shall determine whether there is maternal toxicity and/or toxicity to the pups in order to provide a basis for determining the doses for the subsequent toxicity study (90-day or 2-year study). The animals shall be exposed to the test article during *in utero* development, through their mother's milk, and via dosed feed, dosed water, or gavage administration until PND21.

## B. STUDY DESIGN

### 1. GESTATION PERIOD

Timed-pregnant, approximately gestation day 2 (GD2), female Wistar Han rats shall be approximately 9-10 weeks of age upon receipt. Each female shall be housed individually and maintained on NIH-07 diet throughout the range finding study. All dams and pups shall remain in quarantine status during the study. On GD5 eight dams shall be randomly assigned to each of five treatment and vehicle control groups and uniquely identified by tail tattoo. Starting on GD6 and continuing until postnatal day (PND) 21 the dams shall be gavaged daily (including weekends and holidays) or provided test article in feed or drinking water ad libitum. For gavage studies on the morning of the expected day of delivery if dams are not in the process of delivering then the dams shall be dosed; if in the process of delivery then wait 24 hours. Mortality shall be reported weekly during gestational dosing period for gavage studies. Dams shall be weighed on GD5 prior to dosing, and then on GD6, 9, 12, 15, 18 and 21 for dosed water and dosed feed studies. Starting on GD9 food or water consumption shall be measured every three days during gestation. For gavage studies dams shall be weighed on GD5 prior to dosing and then daily throughout the gestation period to calculate dose. All dams shall be observed two times daily, once in the early morning and once in the late afternoon, at least six hours apart (before 10:00 AM and after 2:00 PM), including holidays and weekends, for signs of delivery, moribundity, and death. Dams that do not deliver within 3-4 days of anticipated delivery date shall be euthanized, examined for uterine implantations/resorptions, and these findings recorded.

| | Pregnant Dams* | | Treatment Groups | | Total |
|---|---|---|---|---|---|
| Treatment | 8 | x | 5 | = | 40 |
| Controls | 8 | x | 1 | = | 8 |
| *Minimum number needed | | | | | 48 |

### 2. LACTATION PERIOD

The lactational period includes the period from delivery until PND21. PND21 is defined as 21 days post delivery of the last litter delivered. Dams shall be weighed on PND1, 4, 7, 14 and 21 for dosed water and dosed feed studies. Food or water consumption measurements are to be made on PND4, 7, 14, and 21 for the periods covered. For gavage studies dams shall be weighed daily throughout the perinatal period to calculate dose. Body weights of individual pups in dosed feed and dosed water studies are to be weighed

at PND4, 7, 14, and 21. For individual pups in gavage studies, body weights are to be taken at PND4, 7, 12, and daily thereafter until PND21. All dams and pups shall be observed two times daily, once in the early morning and once in the late afternoon, at least six hours apart (before 10:00 AM and after 2:00 PM), including holidays and weekends, for moribundity and death and for abnormal maternal behavior.

3. PND1

The number, sex, and general appearance of the pups shall be recorded the day after delivery (PND1). Body weights shall be recorded for dams and litter weights for pups by sex.

4. PND4

Pups shall be culled on PND4 to a maximum of eight per litter (randomly selected to 4 males and 4 females if possible). Each selected pup shall be tattooed (on a limb) for unique identification.

| | Pups | | Treatment Groups | | Sex | | Total |
|---|---|---|---|---|---|---|---|
| Treatment | 32 | x | 5 | x | 2 | = | 320 |
| Controls | 32 | x | 1 | x | 2 | = | 64 |
| | | | | | | | 384 |

5. PND12

For gavage studies, pups shall begin dosing at the same dose as the dams on PND12 and daily thereafter until PND21 (including weekends and holidays). Dam and pup mortality shall be reported weekly during the perinatal dosing period for gavage studies.

6. PND21

At PND21, 21 days after the last litter was delivered, dams and pups shall be sacrificed.

C. OPTIONAL SPECIAL STUDY ENDPOINTS

Additional special studies may be included in the range finding study based on the individual study design requirements. Biosample analysis to include maternal and fetal uptake as well as lactational transfer should be considered. Should organ weights and histopathology be required at PND21 then one male and one female pup per litter shall be randomly selected, sacrificed, and organ weights recorded. Those organs to be weighed are: liver, thymus, right kidney, right and left testis, heart, and lungs. Organs shall be weighed to the nearest 10.0 mg except for testis and thymus which shall be weighed to the nearest 1.0 mg. Complete necropsies are to be performed on the selected pups. All tissues are to be collected, fixed, trimmed, embedded, and slides prepared/stained. All tissues are to be evaluated in the high dose and control pups and then target tissues read to a no-effect-level in lower doses plus any gross lesions.

D. DATA TO BE COLLECTED AND RECORDS TO BE PROVIDED

- Body weights of dams and pups
- Food and water consumption for dams and pups
- Cage observations of dams and pups

- Mortality of dams, pup survival
- Number of dams not littering, number of implantations/resorptions
- Number of dams littering
- Number of male and female pups per litter
- Number and sex of culled pups per litter
- Gavage dosing records for dams and pups

## II. PERINATAL TOXICITY STUDIES (90-DAY OR 2-YEAR)

For standard 90-day studies 12 dams per treatment and control group are required at GD6 and for standard 2-year studies 28 dams per treatment and control group are required. Gestational and lactational exposure shall follow the design of the range finding study. In addition, during gestational and lactational exposure any unexpected toxicity (body weight, mortality, food or water consumption, or clinical signs of dams or pups) that may impact continuation of the gestational/lactational portions of the study or the conduct of the definitive toxicity study shall be reported immediately. All pups shall be weaned on the same day, the day the last litter born reaches PND21. On PND21 2 male and 2 female pups per litter per treatment and control group shall be randomly selected and placed into corresponding treatment and control groups for the subsequent toxicity study. Selected pups are to be either placed on dosed feed or dosed water or dosed by gavage. Fifteen male and female rats not selected from control litters are to be designated as sentinel animals for the 2-year study.

## III. OTHER CONSIDERATIONS

The default species/strain for in utero/lactational studies is Wistar Han rats. Mice are not included. Time-pregnant dams are to be purchased. In-house breeding of dams is not included. Exposure routes shall be limited to gavage, dosed water, and dosed feed. Inhalation and dermal routes of exposure are not included. Fourteen-day toxicity studies are not included for rats.